Supplementary Materials and Methods

Real time quantitative RT-PCR

Collected embryos at various developmental stages were lysed in 10 μl lysis buffer (DEPC-H2O containing 0.1% Triton X-100, 0.1 M DTT and 1 μl RNase OUT Recombinant RNase inhibitor, 5000 U, Invitrogen). 10 μl reverse transcription (RT) reaction buffer containing SuperScript III Reverse Transcriptase (2000 U) (Invitrogen), adapter-Oligo-dT24 (25 nM), RNaseOUT RNase Inhibitor (2000 U) and 0.1 pg \textit{in vitro} transcribed \textit{Egfp} mRNA was then added to the 10 μl lysate. The RT reaction was carried out directly in the tube at 50°C for 1.5 h. RT reaction using \textit{Egfp} specific primer was also carried out in parallel. An aliquot of 1.5 μl of the 20-μl RT reaction was used as template for PCR with gene specific forward primers. The adapter sequence (5'–ATGGATCCGTCCGTCGAC–3') added to the 5’-end of oligo-dT24 (adapter-Oligo-dT24) was used as reverse primer in real time PCR reactions following RT in order to eliminate contamination from mitochondrial genome. Real time quantitative PCR was performed with SYBR Green Mix (Takara) in optical 96-well reaction plates on a CFX96 Realtime System (BioRad). For the examination of 16S mtrRNA levels in microinjected embryos, injected 2-cell embryos were lysed in 10 μl lysis buffer and processed for quantitative RT-PCR with gene specific primers. For single cell quantitative RT-PCR, single blastomeres isolated from 2-cell or 4-cell embryos were placed in 2 μl lysis buffer (DEPC-H2O containing 1% TritonX-100, 10 mM DTT, 1X recombinant ribonuclease inhibitor, TaKaRa, and 1X gDNA buffer, Tiangen) in a PCR tube. Cells were lysed at 70°C for 5 minutes, followed by 42°C incubation for 5 min.
in order to digest the genomic DNA with the DNase contained in the gDNA buffer. 5 μl RT reaction was carried out using AMV reverse transcriptase (TaKaRa) and a combination of oligo-dT₁₈ and 12S or 16S mtrRNA reverse primers for 1 hr at 42°C. RT reaction was used directly for quantitative PCR with gene specific primers without pre-amplification (2.5 μl each for the gene and Actin control, respectively).

**Whole-mount in situ hybridization (ISH) of mouse embryos**

Collected embryos were fixed with 4% PFA (paraformaldehyde) in PBST (PBS with 0.1% Triton X-100) for 15 min, followed by washing 10 min in PBST, 10 min in diluted pre-hybridization solution (1:1 with PBST) and 3 min in pre-hybridization solution (containing 750 mM NaCl, 75 mM NaCitrate, 5X SSC, 10% formamide, 5 mM EDTA, PH 8.0, 0.1% Triton X-100, 50 μg/ml CHAPS, 50 μg/ml heparin). Embryos were incubated in pre-hybridization solution containing 50 μg/ml yeast RNA for 2-3 h, at 65°C. DIG-labeled cRNA antisense or sense probes were then added to pre-hybridization solution to a final concentration of 1 μg/ml and embryos were incubated at 65°C for overnight. After washing twice in pre-hybridization solution at 65°C, 1.5 h each, embryos were incubated in serial dilutions of pre-hybridization solution in PBST (4:1, 3:2, 2:3, 1:4) for 10 min each and twice in PBST, 10 min each. Thoroughly washed embryos were blocked in 2% BSA in PBST for 2 h, followed by incubation with anti-DIG alkaline phosphatase (AP) conjugates (Roche) at 1:2000 dilution in 1% BSA/PBST for 2 h. They were further washed in PBST for 3 times, 15 min each and in Staining buffer (100 mM NaCl, 50 mM MgCl₂, 100 mM Tris-HCl, pH 9.5) for 10 min, before transferred
into Staining buffer containing BCIP/NBT solution. Color reaction was stopped by washing embryos in PBST. Embryos were then mounted onto glass slides in 50% glycerol/PBS and examined with an inverted microscope (Olympus IX71).

**In situ hybridization at electron microscopic level (ISH-EM)**

Isolated embryos were fixed with 4% PFA containing 2.5 mM CaCl₂, 1.25 mM MgCl₂ and 2.9% glucose (fixative vehicle solution was 320 mOsm) dissolved in 0.1 M HEPES-NaOH buffer (pH 7.4) (Fixative 1) for 2 h and then fixed again with 4% PFA containing 2.5 mM CaCl₂, 1.25 mM MgCl₂ and 2.1% glucose (320 mOsm) dissolved in a 0.1 M HEPES-NaOH buffer (pH 8.5) (Fixative 2) overnight at 4°C. *In situ* hybridization was first done with 16S *mtrRNA* long cRNA probes as described above. Hybridized embryos were fixed again with Fixative 2 for overnight, then dehydrated sequentially on ice in: 50% DMF (N, N-Dimethylformamide, Fluka), 15 min; 70% DMF, 15 min; 90% DMF, 15 min and 100% DMF twice, 15 min each. Embryos were then infiltrated with DMF and LR-White resin mixture at ratios of 2:1 and 1:2, respectively, for 30 min each. They were further incubated with fresh LR-White for overnight at 4°C. Embryos were infiltrated with fresh LR-White for another 2 h before transferred into gelatin capsules filled with resin and polymerized for 44 h under UV light using benzoin ethylether as a catalyst at 4°C. Thin (100 nm) sections were cut with an Ultramicrotome (Leica Instruments) and mounted onto nickel grids. Grids were washed with ddH₂O and TBS (0.15 M NaCl, 20 mM Tris–HCl, pH 7.4) for three times each and then blocked with 1% BSA/TBS for 30 min. Mouse monoclonal anti-DIG antibody (1:200, Sigma) diluted in 1% BSA/TBS was then used to incubate
grids for overnight at 4°C. Following washing with TBS, grids were incubated with goat anti-mouse IgG conjugated with 12-nm colloidal gold (1:20, Jackson) for 1 h. Grids were then washed with TBS and ddH₂O before being fixed again with 2% glutaraldehyde containing 0.05% tannic acid in 0.1 M PBS, pH 5.5, for 5 min. After washing with ddH₂O for 3 min, grids were incubated with 1% OsO₄/0.1 M PBS (pH 7.4) for 5 min and washed again with ddH₂O for 3 min. Grids were then stained with saturated uranyl acetate aqueous solution for 20 min and Reynold’s lead citrate solution for 8 min and washed with ddH₂O for 3 min.

**Transmission electron microscopy (TEM)**

For transmission electron microscopy without ISH, embryos were fixed in 3% glutaraldehyde (SPI Supplies, USA) for 1 h, and then with 1% Osmic Acid (Electron Microscopy Sciences, EMS, USA) for 90 min. The Embryos were dehydrated in an acetone gradient and embedded in Epon812 resin (TED PELLA INC., USA). Thin (100 nm) sections were cut with a LKB2188 microtome (LKB BROMMA, Sweden), transferred to 200 mesh carbon-coated copper grids, stained with 2% uranyl acetate for 30 min and Reynold’s lead citrate for 10 min. Stained samples were examined using an electron microscope (Tecnai G2 Spirit, FEI).

**Fluorescent in situ hybridization (FISH)**

Mouse embryos were collected from superovulated CD1 mice and fixed in 4% PFA/PBS for 20 min at R.T.. Embryos were then washed with PBS twice, 10 min each and permeabilized in 70% ethanol/PBS overnight at 4°C. Permeabilized embryos were re-hydrated with serial dilutions of ethanol in
PBS till 0.5% ethanol/PBS, washed in 1X PBS for 5 min and incubated in washing buffer (10% formamide, 2X SSC in DEPC H$_2$O) for 5 min at R.T.. They were then incubated in pre-warmed hybridization buffer (10% formamide, 2X SSC, 100 mg/ml dextran sulfate in DEPC H$_2$O) containing 125 nM Stellaris probes, for 6-8 hours in the dark at 37°C. In some cases, both sets of probes were used simultaneously. Following hybridization, embryos were washed with pre-warmed washing buffer for 30 min and stained with DAPI (4', 6-diamidino-2-phenlindole, 0.5 μg/ml, Molecular Probes) for 5 min and washed again for 30 min at 37°C. Stained embryos were then mounted onto glass slides in Vectashield mounting medium (Vector Laboratories, Inc., H-1000) and examined with Confocal Laser Scanning Microscopy (CLSM, LSM 710 NLO, Carl Zeiss).

**Immunofluorescent staining of mouse embryos**

To detect mitochondria content, mouse embryos were stained with MitoTracker Red (200 μM, Molecular Probes). Live embryos were incubated in KSOM media (containing 200 μM MitoTracker Red, Molecular Probes) for 30 min at 37°C. After staining, embryos were washed 3-4 times with KSOM and fixed with 4% PFA/PBST for 15 min. They were then washed and stained with DAPI for nuclei for 3 min and mounted onto glass slides. In some cases, stained embryos were further processed for fluorescent *in situ* hybridization of mtrRNAs using Stellaris probes according to the procedures described above.

To detect mitochondria activity, live embryos were incubated in 50 μl of pre-warmed M2 media containing tetramethyl rhodamine methyl ester (TMRM, 25 μM, Molecular Probes) and 10 μg/ml Hoechst 33342 for 30 min at
37°C. Embryos were washed with M2 containing lower concentration of TMRM (1:50000) for 30 min at 37°C and observed in the same media using CLSM. In some cases, mitochondria membrane potential was un-coupled with protonophore carbonyl cyanide p-trifluoromethoxyphenyl-hydrazone (FCCP, 5 mM in pre-warmed M2) briefly for 5 sec before TMRM staining.